Processes Affecting the Variability of Fluorescence Signals from Benthic Targets in Shallow Waters

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LONG-TERM GOAL

Our major theme is to understand and to qualify processes that contribute to fluorescence emission from benthic targets in the coastal and shallow waters with the overarching goal of developing parametrization schemes that optically detect anthropogenic objects.

SCIENTIFIC OBJECTIVE:

1. To identify the sources of variations in the effective absorption cross sections of the target molecules, fluorescence lifetimes (and by inference, quantum yields) of individual chromophores, and to provide an interpretive understanding of how physical, chemical, and biological variability affects these optical properties.
2. To determine the extent and variability in the coupling of absorbed radiation to the fluorescence emission spectrum, and the development of biophysical radiative transfer models that predict the latter from the former in a variety of benthic environments.
3. To develop an understanding of the spatial and temporal variability in benthic and optical signals.

APPROACH

We applied two basic techniques to study variability in fluorescence signals from benthic targets: Fast Repetition Rate (FRR) Fluorometry (Falkowski P.G. and Kolber Z. 1995; Kolber et al, 1998; Prasil et al., 1998) and time-resolved picosecond measurements of fluorescence lifetimes (Holzwarth, 1986). We developed a new version of the scuba-based FRR fluorometer (SCUFFR). The initial data set acquired by the first prototype SCUFFR in the Dry Tortugas indicated that simultaneous fluorescence data acquisition and image capture of the target was desirable. Hence, we developed a second version of the SCUFFR to achieve these goals. Specifically, we modified the optical design of the excitation/emission channel and increased sensitivity five-fold thereby greatly increasing the signal-to-noise ratio. The flash intensity was automatically adjusted with a feedback circuit using DSP-based electronics to control an excitation sequence of FRR flashes and the acquisition of fluorescence signals. A compact CCD video camera was incorporated into the instrument, allowing the user to monitor a target in real time and to acquire its image simultaneously with FRR fluorescence measurements. Finally,

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orthogonal, off axis long wave laser diodes were incorporated in the view finder to precisely control the distance to the target. Data is stored on-board in a flash card and downloaded following a dive. The instrument can store several hundred profiles. In conjunction with the SCUFRR instrument, a bench-top FRR fluorometer was used for detailed laboratory analysis of zooxanthellae isolated from coral samples.

We examined fluorescence lifetimes from benthic targets using femtosecond laser based Single Photon Counting Time-Correlated Fluorometry (in the lab) and Phase Shift Fluorometry (during CoBOP-98 field studies). Whereas the first technique permits precise analysis of multi-component kinetics in the laboratory, the second approach is suitable for rapid routine measurements of the average fluorescence lifetimes in the field. In the first technique, 50 fs pulses from Ti:Sapphire mode-locked laser are used to induce fluorescence. The signal is detected by an ultra-fast MCP photomultiplier and processed with a discriminator and a time-to-amplitude converter interfaced to a Multichannel Analyzer. With excitation at 400 nm, the laser set-up allows analysis of the decay kinetics of fluorescence throughout the entire visible region. The accumulation of fluorescence decay profiles with a sufficient signal-to-noise ratio permits sophisticated multi-component analysis of the decay kinetics with a temporal resolution 5 ps. The laser set-up was employed for laboratory studies of fluorescence lifetimes from model targets such as zooxanthellae, as well intact coral samples. During the CoBOP field campaign on LSI, a portable Phase Shift Fluorometer, from Ciencia, was used for measuring average lifetimes of red, orange and green fluorescence from coral samples.

WORK COMPLETED

The new SCUFRR fluorometer was employed during the CoBOP-98 field campaign at Lee Stocking Island and was used to study a wide variety of benthic targets. Over one thousand six hundred measurements were made on corals, both *in-situ* and from experimental systems at LSI; 80 measurements were made on surficial sediments, and 25 measurements on samples of seagrasses. Over 200 measurements on isolated zooxanthellae were conducted with a bench-top FRR fluorometer and Phase Shift Fluorometer. During the CoBOP-98 field campaign, we particularly focused on the study of diel variability in bio-optical properties of corals and seagrasses. Thirty-two samples of corals *Montastreae faveolata* and *Montastreae cavernosa,* and seagrasses were investigated during a 7 day long time series. To simulate various light regimes, the samples were placed in shaded and non-shaded tanks and, thus, exposed to various levels of ambient irradiance. Initial data analysis has been completed and files for the database were prepared. With the use of the picosecond time-resolved laser set-up, a series of laboratory experiments were completed to study fluorescence lifetimes from model targets such as zooxanthellae cultivated from *Aiptasia*, *Porites, Montipora, Cassiopea,* and *Zoanthus,* as well intact coral samples. Our particular emphasis was given to the study of variability in fluorescence lifetimes due to changes in the functional state of PSII, as well as varying environmental factors such as nutrient availability and ambient irradiance.

RESULTS

Application of the Scuba FRR Fluorometer to study optical properties of benthic targets.

Fig. 1 shows typical SCUFRR fluorescence profiles measured on coral (Montastraea faveolata), sediment, and seagrass. Table 1 summarizes average fluorescence intensities (F_o and F_m levels) and photosynthetic parameters, calculated from the FRR profiles, for various groups of benthic targets.

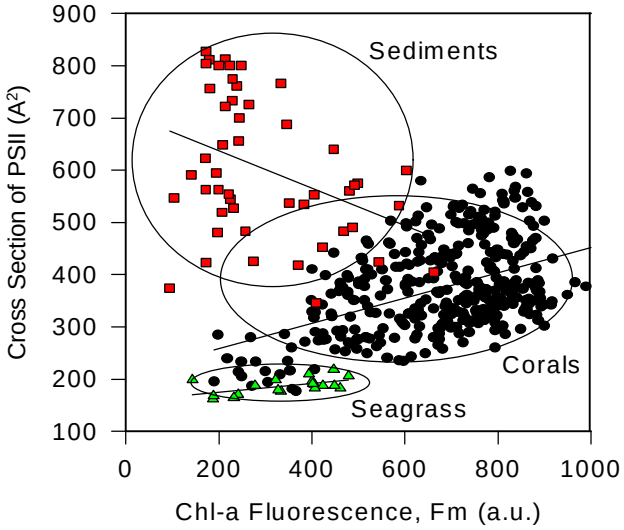
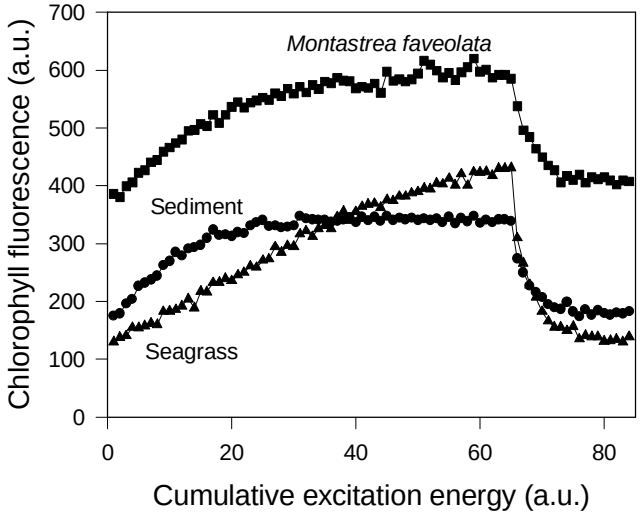
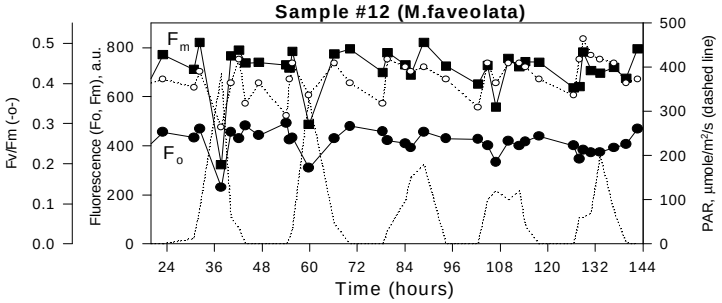


Table 1. Averaged fluorescence intensities and photosynthetic parameters for various groups of benthic targets measured with the scuba based FRRF.

	Corals (380 samples)	 Algal turf and films on sediments (49 samples)	Seagrasses (20 samples)
F_o	440 ± 100	150 ± 70	122 ± 25
F_m	720 ± 140	300 ± 140	390 ± 70
F_v/F_m	0.39 ± 0.07	0.50 ± 0.05	0.70 ± 0.04
σ_{PSII}	395 ± 80	605 ± 135	190 ± 15

Different targets were found to exhibit different fluorescent and photosynthetic signatures. Corals

possess relatively low quantum yields of photochemistry in PSII: F_v/F_m averages 0.39, i.e. 60% of its maximum value $(F_v/F_m)^{max}$ = 0.65 typical for zooxanthellae growing under optimal, nutrient-replete conditions. Low photochemical activity and high chlorophyll fluorescence lifetimes (typically 1-1.2 ns) suggest nutrient limitation of zooxanthellae in symbiotic corals. Algal turf on sediments, comprising dinoflagelates and diatoms, exhibited relatively high σ_{PSII} and elevated photochemical activity: F_v/F_m = 0.50 (70-80% of its maximum value). The intensities of chlorophyll fluorescence from sediment surfaces vary from 0 (no algae on the surface) to the values close to, or even exceeding, those from seagrasses. In contrast to corals and sediments, seagrasses possess low σ_{PSII} and maximum quantum yields of photochemistry in PSII: F_v/F_m was found to be 0.70 $\pm$ 0.04, indicating absence of nutrient limitation. Note that the SCUFRR fluorescence data demonstrate that the intensity of chlorophyll fluorescence (at F_o level) from seagrasses leaves is in average lower than that from sediments and corals. These SCUFRR fluorescence data provide some clues to interpreting the fluorescence images obtained with the Laser Line Scanner; the latter shows very low visibility (in the red channel) of seagrasses on the background of sand and sediments.

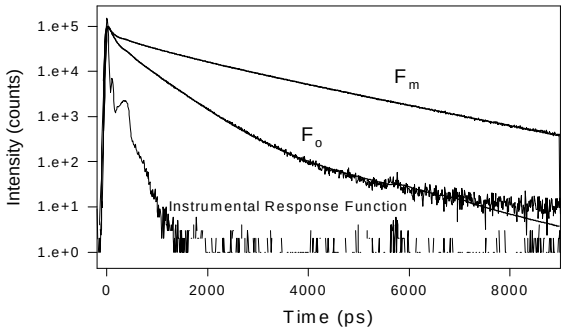


Differences in bio-optical and photosynthetic signatures, measured with the SCUFRR fluorometer potentially permits the development of algorithms for discrimination and identification of different benthic targets. For example, high yields of variable fluorescence in conjunction with low σ_{PSII} was found to be a unique signature of seagrasses that can be used to identify seagrasses on the background of other fluorescing benthic targets as sediments, algal turf, and corals. Fig. 2 demonstrates how different targets can be identified using SCUFRR fluorescence measurements of F_m and σ_{PSII}. One of our goals within the framework of this project is development of algorithms for identification of different benthic targets based on optical signatures retrieved with the SCUFRR fluorescence technique.

Temporal variability of fluorescence yields in benthic targets.

One of the key problems related to interpretation of fluorescence measurements is high variability in the quantum yields of *in-vivo* chlorophyll fluorescence. Being a by-product of photosynthetic processes in PSII, *in-vivo* chlorophyll fluorescence is affected by the functional state of photosynthetic apparatus. As a consequence, the quantum yield of *in-vivo* chlorophyll fluorescence varies up to 3-5 times depending on the environmental conditions as nutrient availability, ambient irradiance, presence of anthropogenic toxins, etc. The fluorescence yield of chlorophyll in marine phytoplankton can vary up to four-fold within a diel cycle. Diel variability in fluorescent properties of benthic organisms is, however, not well documented.

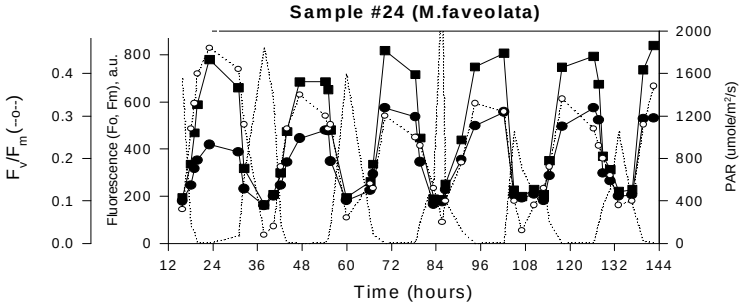
Fig. 3 shows diurnal variations in fluorescence yields and photosynthetic parameters in several samples of *M. faveolata*. The main results obtained from the studies of diel variability of



chlorophyll fluorescence and photosynthetic activity are as follows:

a. Chlorophyll fluorescence is quenched by irradiance in corals. The diel variability is up to 4-fold and is a non-linear function of irradiance.

b. Diel variations in the intensity of chlorophyll fluorescence are caused by appropriate variations in the quantum yield of chlorophyll fluorescence, whereas changes in the absorption properties and chlorophyll concentration do not contribute markedly to diel variability of fluorescence.



c. Non-photochemical quenching is associated primarily with non-radiative deactivation of excited states in the antenna and is directly correlated with a decrease in the functional absorption cross section of PSII.

d. Non-photochemical quenching of chlorophyll fluorescence is higher in nutrient-limited samples with elevated F_v/F_m ratios

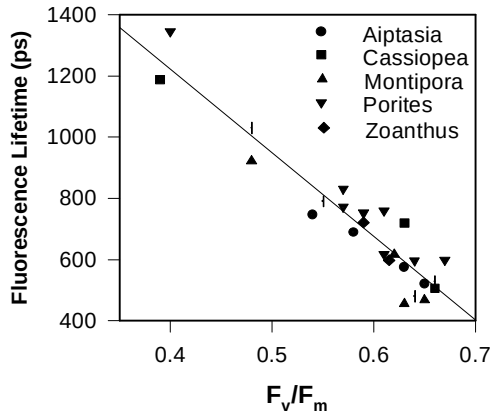
e. Seagrasses are less susceptible to non-photochemical quenching than corals.

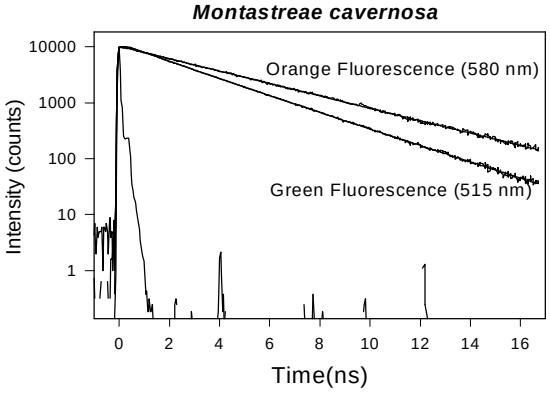
Summary on the Study of Fluorescence Lifetimes of Benthic Targets:

a. Decay kinetics of Chl-a fluorescence from corals can be decomposed into 4 components with the lifetimes ranging from 25 ps to 2-3 ns (Table 2).

b. When all the reaction centers of PSII are active and open, the medium component (400-600 ps) is dominant; closing the reaction centers results in an increase in the yield of the slow component which becomes dominant while all the centers are closed (Fig. 4).

c. The average lifetime of chlorophyll fluorescence in for open reaction centers (Fo) can be directly related to the changes in variable fluorescence retrieved by the SCUFRR fluorescence measurement; the lower the ratio F_v/F_m (i.e. the quantum yield of photochemistry in PSII), the longer is the lifetime of chlorophyll fluorescence (Fig. 5).





d. The relationship between lifetimes of chlorophyll fluorescence and F_v/F_m suggests that the F_v/F_m ratios, measured rapidly and *in-situ* with the SCUFRR fluorescence technique, can be used to assess the intrinsic lifetimes of chlorophyll fluorescence and, thus, the quantum yields of chlorophyll fluorescence.

e. Decay kinetics of orange fluorescence (emission maximum at 580 nm) includes 2 components with the lifetimes 130 ps and 3930 ps (Fig.6, Table 3). The dominating long component (3930 ps, 99%) originates from phycoerythrin-containing phycobiliproteins that are not connected to PSII reaction centers; the minor fast component is attributed to phycobiliproteins connecting to PSII centers and, thus, participating in the harvesting of radiative energy and transferring the resulting excitation energy to PSII .

f. Decay kinetics of green fluorescence (emission maximum at 515 nm) consists of 2 components with the lifetimes 1875 ps and 3010 ps (Fig.6, Table 3).

g. There is no excitation coupling between blue-green fluorescing compounds and PSII photochemistry. By implication, the variability of the fluorescence yields of these components will be small.

Table 2. Lifetimes (τ_i) and relative yields (f_i) from the 4-component analysis of Chl-a fluorescence decay for minimum (F_o) and maximum (F_m) levels; the quantum yields of fluorescence (ϕ) were assessed from the average lifetimes and absorption properties of

chlorophyll.

F_o		F_m	
τ_i (ps)	f_i	τ_i (ps)	f_i
25	7.3%	25	1.3%
167	8.1%	117	3.2%
504	**82.5%**	694	18.3%
1595	2.1%	**1285**	**77.1%**
$\tau_{average}$ = 465 ps		$\tau_{average}$ = 1123 ps	
ϕ = 1.8 %		ϕ = 4.5 %	

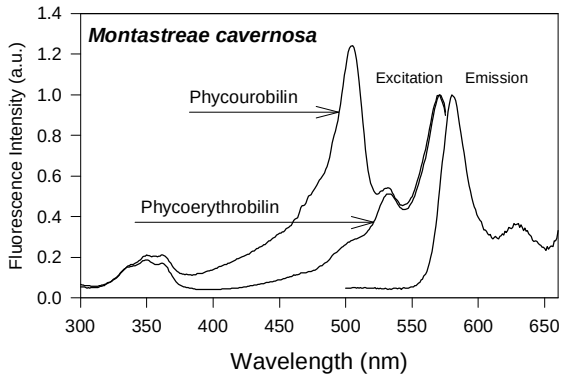
Table 3. **Lifetimes (τ_i) and relative yields (f_i) from the 2-component analysis of fluorescence decay.**

Orange Fluorescence (580 nm)		Green Fluorescence (515 nm)	
τ_i (ps)	f_i	τ_i (ps)	f_i
130	0.7%	1876	14%
3930	99.3%	3010	86%
$\tau_{average}$ = 3930 ps		$\tau_{average}$ = 2850 ps	

Origin and properties of orange fluorescence from corals.

As a part of the CoBOP field studies, we investigated time-resolved and spectral features of orange and green fluorescence from corals. Those studies were conducted in close collaboration with Charlie Mazel and Michael Lesser. The results suggest that the orange fluorescence originates from phycoerythrin-containing cyanobacteria living in symbiotic corals. This inference was developed from the following:

- the striking similarity of excitation/emission spectral properties of the orange fluorescence from corals with those of phycoerythrins;
- epifluorescent microscopic identification of cyanobacteria in orange-fluorescing corals;
- time-resolved studies of the orange fluorescence.



We found that, as in planktonic cyanobacteria and red algae (Glazer A.N. et al., 1982; Glazer A.N., 1985), orange-fluorescing corals have two types of phycobiliproteins, namely, R-phycoerythrins, those are rich of phycourobilin (PUB), and B-phycoerythrins lacking of PUB. These phycobiliproteins exhibit identical fluorescence emission spectra (Fig. 7), the same lifetimes of fluorescence, but different fluorescence excitation spectra (Fig. 7).

Variability in the quantum yields of fluorescence from phycobiliproteins is primarily caused by changes in the efficiency of excitation transfer from these accesory chromophores to chlorophyll-a.
Because the phycobiliproteins are the major light harvesting antenna systems in cyanobacteria, there should be excitation coupling between phycobilins and PSII photochemistry. Initial data collected during the CoBOP field campaign suggest, however, that the extent of this coupling is very small. The long lifetime component of the orange fluorescence and, by inference, high quantum yield of fluorescence (about 40-50%) in high-fluorescing corals *M.cavernosa* (Table 3) suggests virtually no excitation coupling between phycobiliproteins and PSII. However, spectral resolved fluorescent studies in some fluorescing species suggest marked excitation coupling (C.Mazel, personal communication). We hypothesize that the extent of excitation coupling will be higher, and, thus, the quantum yield lower in low fluorescing corals. Theoretically, the quantum yield of orange fluorescence may vary by an order of magnitude in this case, and should be taken into account in RTE's.

IMPACT/APPLICATION

Understanding the sources of variability and behavior of benthic fluorescent targets, such as corals, turf algae, invertebrates, and seagrasses, is essential to developing operational protocols for distinguishing between anthropogenic and naturally occurring objects. Moreover, within the overall goals of CoBOP, namely identification and quantitation of IOPs that are required for closure of radiative transfer models, fluorescence is a source of spectrally camouflaged photons that are radiated from benthic targets that have absorbed photons at other wavelengths. Thus, consideration of varying fluorescence processes in conjunction with the measurements of spectral absorption and scattering will lead to numerically accurate and complete radiative transfer models. In addition, the set of fluorescent and photosynthetic signatures of benthic targets acquired by the SCUFRR fluorometer is going to be applied for interpreting the fluorescence images obtained with the Laser Line Scanner. correction of measuring reflectance in the red spectral region

TRANSITIONS

Our SCUFRR fluorometers have been used by Dr. Michael Lesser in an ONR-funded study of the effect of spectral quality and quantity if the underwater light field and elevated temperatures on

small scale optical properties of corals. The SCUFRR instrument will be also used by Professor Z. Dubinski and his colleagues (Bar-Ilan University, Ramat Gan, Israel) for studying coral reefs in the Red Sea.

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